Attorney Docket: /51594US
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	ANDERS BERKENSTAM ET AL		
Serial No.:	09/896,791	Group Art Unit:	1642
Filed:	June 29, 2001	Examiner:	Nickol, Gary B.
Title:	SCREENING METHODS		

DECLARATION UNDER 37 C.F.R. 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Lorenz Poellinger, Ph.D., declare and state that:

1. I am a citizen of Sweden/Germany.

2. I am currently a professor of the Department of Cell and Molecular Biology in the Karolinska Institutet, Stockholm, Sweden.

3. I have a Ph.D. in __Biochemistry/Molecular Biology__from Karolinska Institute, Stockholm, Sweden____(a Medical University).

4. I have __21___ years of experience in the field cell and molecular biology, and since __1996_____. I have been teaching and conducting research related to mammalian angiogenesis. A copy of my Curriculum Vitae is attached to show in more details my technical expertise.

5. I am an inventor of the above-referenced application. The application discloses, *inter alia*, a polynucleotide molecule, as represented by SEQ ID NO:

2, that encodes an Inhibitory PAS Domain Protein (IPAS), as represented by SEQ ID NO: 3. The application also discloses "stringent hybridization conditions" under which another polynucleotide molecule remains hybridized with SEQ ID NO: 2.

6. It takes only minimal effort and skills for anyone with general training in molecular biology to express a polynucleotide molecule that remains hybridized under stringent condition with SEQ ID NO: 2, to obtain a polypeptide encoded by the polynucleotide molecule.

7. The polypeptide so obtained ("candidate polypeptide") can be easily and quickly tested to determine if it has an IPAS activity, for example, employing a transient transfection assay.

8. Specifically, human HeLa epithelial cells can be co-transformed (e.g. by transient transfection) with a hypoxia-response element-(HRE-) driven luciferase reporter gene construct and a vector that expresses the candidate polypeptide, and both the candidate polypeptide and the reporter are co-expressed. If the candidate polypeptide does not possess IPAS activity, incubation of the cells under hypoxic (1% O_2) conditions results in induction of the reporter gene activity, reflecting the induced transactivation function of endogenous hypoxia-inducible transcription factors (HIFs). If the candidate polypeptide possesses IPAS or IPAS-like activities, a reduction of the hypoxia-inducible reporter gene activation response will result.

9. The above procedure is routine and can be performed by a lab technician or a graduate Ph.D. student. The materials necessary are readily available. For example, HeLa cells can be obtained from ATCC (American Tissue Culture Collection). Transient transfections are easily carried out by the lipofection procedure in 28 cm^2 culture plates. In luciferase reporter gene assays, 0.5 μg of reporter plasmid can be used for transfection together with increasing amounts of expression vector (e.g. plasmids) encoding the candidate polypeptide. Conditions for hypoxia treatment of the cells are well known and are described previously (Gradin, K. *et al.* Functional interference between hypoxia and dioxin signal transduction pathways: competition for recruitment of the Arnt transcription factor. *Mol Cell Biol* 16, 5221-5231 (1996)).

10. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

July 11, 2003
Date

Lorenz Poellinger
Lorenz Poellinger, Ph.D.

CURRICULUM VITAE
LORENZ POELLINGER

Date of birth: Jun 7, 1957
Place of birth: Stockholm, Sweden

PRESENT POSITION

Professor of Molecular Biology; Dept. of Cell and Molecular Biology, Karolinska Institutet, 171 77 Stockholm, Sweden
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EDUCATION

Year	Institute	Degree
1980	Karolinska Institutet, Stockholm	Bachelor of Medicine
1985	Karolinska Institutet	Ph.D.
1987-1989	Rockefeller University, New York, NY, Dept. Biochem. & Mol. Biol. (Prof. Robert G. Roeder)	Post.doctoral studies as a senior research associate
1991	Karolinska Institute	M.D.

ACADEMIC POSITIONS AND FELLOWSHIPS

Research associate, Dept. of Medical Nutrition, Karolinska Institutet, 1985-1986.

Research Fellowship (research associate), Swedish Medical Research Council, Dept. of Med. Nutrition, Karolinska Institutet, 1986-1990

Senior Research Associate, Rockefeller University, New York, NY (see above), 1987-1989

Acting Senior Lecturer in Medical Nutrition, Dept. of Med. Nutrition, Karolinska Institutet, 1989-1991

Tenure, member of Faculty, Senior Lecturer in Cell and Molecular Biology, Karolinska Institutet, Dept. of Med. Nutrition , 1991-1996.

Professor in Molecular Biology, Dept. of Cell & Molecular Biology, Medical Nobel Institute, Karolinska Institutet, 1996-present

SCIENTIFIC AWARDS

-Anders Jahre Prize for Young Medical Investigators, Oslo, 1995

-T. Swedberg Prize, The Swedish Society for Biochemistry and Molecular Biology, Stockholm, 1996

SCIENTIFIC JOURNAL COMMITMENTS

Member of Editorial Boards: Molecular Pharmacology, (ASPET: American Association of Pharmacology and Experimental Therapeutics); European Journal of Pharmacology and Toxicology; Journal of Biochemistry, Tokyo.

SUPERVISION OF Ph.D. THESES

Supervisor of 11 successfully defended Ph.D. theses

SUPERVISION OF POSTDOCTORAL RESEARCH

Supervisor of 9 postdoctoral research fellows.

INVITED SPEAKER TO MAJOR INTERNATIONAL CONFERENCES

Invited speaker to about 90 international conferences or symposia.

Special Competence:

Key words:

Signal transduction; Gene Expression; Gene Regulation, Gene Transcription; Transcription Factors; Cell Stress; Oncogenes; Angiogenesis; Vascular Biology; Chemical Carcinogenesis; Drug Metabolism